

Vegetables, Fruits, and Carotenoids and the Risk of Cancer

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Carotenoids are naturally occurring pigmented compounds, usually yellow to red in color, that are abundant in plants and are introduced into animal tissues through dietary intake. To date approximately 600 distinct carotenoids have been identified, approximately 10% of which are considered to be "provitamin A" because they can be metabolically converted to vitamin A in humans (Olson, 1989). Beta-carotene is the most abundant of the provitamin A carotenoids in the U.S. food supply. In addition to their role as vitamin A precursors, carotenoids also have antioxidant and immunological properties which may be important in cancer etiology and prevention (reviewed in Bendich and Olson, 1989).

POSSIBLE ROLES IN CANCER PREVENTION

Several mechanisms have been proposed whereby carotenoids may directly play a role in cancer prevention. The most widely discussed is their antioxidant potential. Highly reactive free radicals and singlet oxygen can damage cell membranes and genetic material. Carotenoids may prevent oxidative degradation, which has been proposed as a step in the development of malignancy (reviewed in Burton, 1989). It should be noted, however, that antioxidant properties are shared by other dietary constituents, such as vitamins C and E. In addition, an immunological role has been proposed for carotenoids. There is preliminary evidence that carotenoids may enhance the immune response responsible for

killing cancer cells, possibly by protecting macrophages, natural killer cells, and cytotoxic T cells from oxidation (reviewed in Bendich, 1989).

STRUCTURE

Most of the naturally occurring carotenoids possess 40 carbon atoms. More than 600 have been identified, not including cis-trans and optically active isomers (Britton and Goodwin, 1982). Most of the carotenoids in food or human blood are hydrocarbon carotenoids, such as beta-carotene, alpha-carotene, and lycopene, or C⁴⁰ xanthophylls, such as cryptoxanthin, lutein, and zeaxanthin (Straub, 1987). Figure 1 presents the chemical structures of the carotenoids abundant in food and in animal tissues.

MEASURING THE CAROTENOID CONTENT OF FOODS

Originally carotenoids were considered important in nutrition because of their vitamin A activity. Only in the last 15 years has there been significant interest in evaluating carotenoids for roles unrelated to their conversion to vitamin A. Thus, food composition tables in the U.S. present vitamin A and not carotenoid values for individual foods (USDA, 1976-1987). Vitamin A activity in these tables was first expressed in international units (IU); in 1967, the FAO/WHO Expert Group recommended that retinol equivalents (RE) be used (FAO/WHO Expert Group, 1967). REs take into consideration the inefficient utilization of dietary carotenoids as vitamin A relative to dietary retinol (Committee on Dietary Allowances, 1980). Both poor absorption of carotenoids and limited metabolic conversion to vitamin A are involved. Table 1 lists the estimated vitamin A activity of selected retinoids and carotenoids using all-*trans*-retinol as the standard (Beecher and Khachik, 1984). It is obvious that using the vitamin A activity in REs as the sole index of the total carotenoid content of foods leads to an underestimate of the carotenoids actually present, since most carotenoids have no provitamin A activity.

As interest increased regarding the precise carotenoid content of foods, investigators made reasonable assumptions about the vitamin A values listed in food composition tables. The vitamin A activity assigned to fruits and vegetables was assumed to be entirely from provitamin A carotenoids. Formulas were derived to calculate carotenoid content, based on the poor utilization of various carotenoids as vitamin A: 1 RE of vitamin A = 6 μ g beta-carotene or 12 μ g of other provitamin A carotenoids = 1 μ g retinol. Such calculations were reasonable for estimating beta-carotene, the most abundant provitamin A carotenoid in foods, but were less appropriate for other provitamin A carotenoids and ignored the carotenoids without vitamin A activity (Beecher and Khachik, 1984).

Very few food composition tables currently provide carotenoid values directly

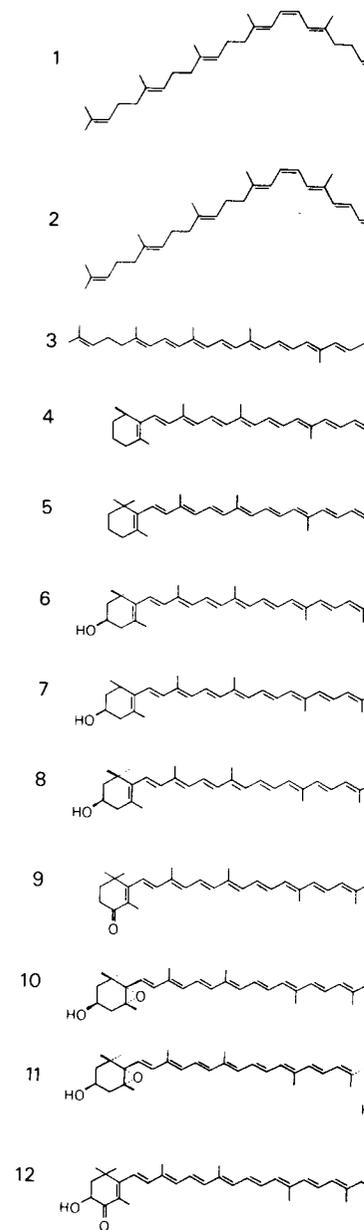


Figure 1 Polyenes and carotenoid: animal tissues. 1, phytoene; 2, phytofluene; 5, beta-carotene; 6, beta-cryptoxanthin; 10, violaxanthin; 11, neochrome (Beecher and Olson, 1989.)

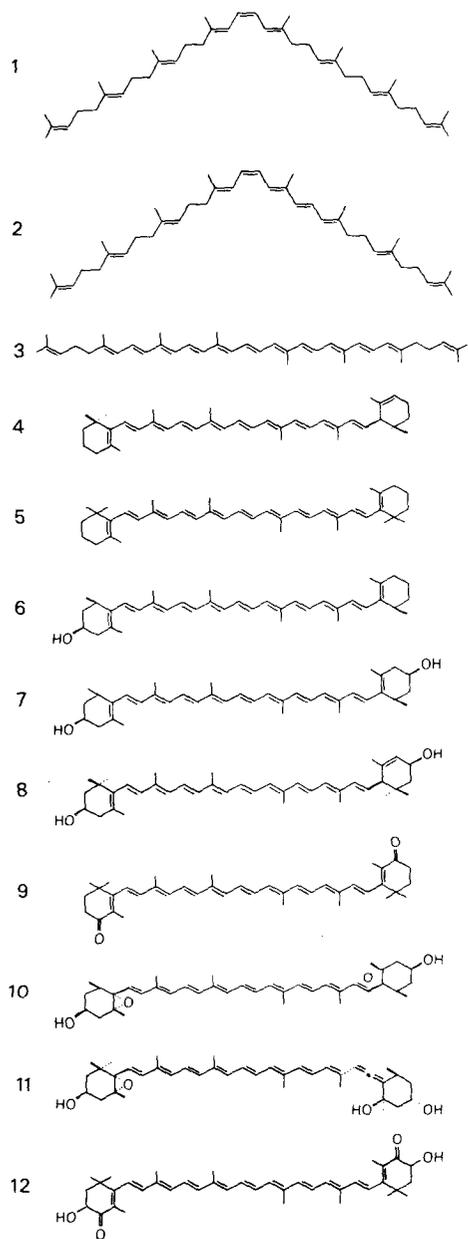


Figure 1 Polyenes and carotenoids in foods that may also be found in animal tissues. 1, phytoene; 2, phytofluene; 3, lycopene; 4, alpha-carotene; 5, beta-carotene; 6, beta-cryptoxanthin; 7, zeaxanthin; 8, lutein; 9, canthaxanthin; 10, violaxanthin; 11, neoxanthin; 12, astaxanthin. (From Bendich and Olson, 1989.)

Table 1 Vitamin A Activity of Various Naturally Occurring Retinoids and Carotenoids

Retinoid or carotenoid	Vitamin A activity
Retinoids	
All- <i>trans</i> -retinol	100
11- <i>cis</i> -retinol	75
13- <i>cis</i> -retinol	85
Carotenoids	
All- <i>trans</i> -beta-carotene	17
9- <i>cis</i> -beta-carotene	6
All- <i>trans</i> -beta-carotene	8-9
Lycopene	0
Beta-cryptoxanthin	8-10
Lutein	0
3-keto- β -carotene	9
Beta-carotene-5,6-epoxide	3-4
Beta-apo-8'-carotenal	12
Beta-apo-10'-carotenal	0

Source: From Beecher and Khachik, 1984.

(Beecher and Khachik, 1984). The U.S. Department of Agriculture's Revised Handbook 8 (USDA, 1976-1987) presents vitamin A data for vegetables and fruits based on the Association of Official Analytic Chemists methods (Horwitz, 1980). The AOAC methods only separate major classes of compounds, such as hydrocarbon carotenoids and xanthophylls, and do not separate individual carotenoids. State-of-the-art analyses of the carotenoid content of foods are now being performed by USDA in conjunction with the National Cancer Institute using high-performance liquid chromatography (HPLC). Such procedures are able to quickly separate and quantitate alpha- and beta-carotene, lycopene, and some individual xanthophylls, such as lutein and beta-cryptoxanthin (Beecher and Khachik, 1989).

As updated carotenoid values become available, they are published in the scientific literature and used to update the vitamin A values in USDA Revised Handbook 8. Footnotes indicate when the vitamin A values are based on HPLC methods (Beecher and Khachik, 1984). Most investigators, however, have not had the opportunity to utilize updated carotenoid data in evaluating the carotenoid content of the diet and in investigating the role of beta-carotene, other individual carotenoids, and carotenoids in general in the etiology of cancer.

In a recent study (Micozzi et al., 1990) which analyzed by HPLC the carotenoid content of selected foods, fresh green leafy vegetables were found to be moderately high in beta-carotene and very high in xanthophylls, especially lutein. Fresh yellow-orange vegetables, such as carrots, acorn squash, and sweet potato, con-

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tained primarily alpha- and beta-carotenoid without pro

The above study and others sumn that cooking vegetables results in a l and a smaller decrease in total hydro depends on the vegetable and the l geographic area in which produce is ; and Khachik, 1989).

MAJOR SOURCES OF CARC

Table 2 identifies the leading source frequency of food consumption data

Table 2 Food Sources of Vitamin A in The 1987 National Health Interview Sur

Food(s)	% Contrib to total d vitamin A i
Carrots	28.0
Liver	11.1
Sweet potato	9.2
Ready-to-eat cereals	7.8
Milk (fluid)	6.7
Spinach	5.5
Beef stew	5.0
Vegetable soup	3.7
Cheese (hard)	3.0
Margarine	2.9
Collards	2.8
Broccoli	2.6
Salad	2.0
Eggs	1.8
Cantaloupe	1.7
Ice cream	1.3
Spaghetti (incl. sauce and cheese)	1.2
Butter	1.1
Orange juice	1.0
Mayonnaise	0.7

* Derived from values for vitamin A by excluding A values from USDA Revised Handbook 8 (U tomatoes. Data do not represent total carotenoid

tained primarily alpha- and beta-carotene. Tomatoes were rich in lycopene, a hydrocarbon carotenoid without provitamin A activity.

The above study and others summarized by Beecher and Khachik (1989) show that cooking vegetables results in a large decrease in total xanthophylls (25–68%) and a smaller decrease in total hydrocarbon carotenoids (3–12%). The actual loss depends on the vegetable and the length and type of cooking. In addition, the geographic area in which produce is grown affects its carotenoid content (Beecher and Khachik, 1989).

MAJOR SOURCES OF CAROTENOIDS IN THE U.S. DIET

Table 2 identifies the leading sources of vitamin A in the U.S. diet, based on frequency of food consumption data from the 1987 National Health Interview

Table 2 Food Sources of Vitamin A and Beta-Carotene in the U.S. Population: The 1987 National Health Interview Survey

Food(s)	% Contribution to total daily vitamin A intake	Cumulative % of vitamin A	Cumulative % of provitamin A ^a
Carrots	28.0	28.0	43.7
Liver	11.1	39.1	—
Sweet potato	9.2	48.3	58.1
Ready-to-eat cereals	7.8	56.1	—
Milk (fluid)	6.7	62.8	—
Spinach	5.5	68.3	66.7
Beef stew	5.0	73.3	74.5
Vegetable soup	3.7	77.0	80.3
Cheese (hard)	3.0	80.0	—
Margarine	2.9	82.9	—
Collards	2.8	85.7	84.7
Broccoli	2.6	88.3	88.8
Salad	2.0	90.3	91.9
Eggs	1.8	92.1	—
Cantaloupe	1.7	93.8	94.6
Ice cream	1.3	95.1	—
Spaghetti (incl. sauce and cheese)	1.2	96.3	96.0
Butter	1.1	97.4	—
Orange juice	1.0	98.4	97.6
Mayonnaise	0.7	99.1	—

^a Derived from values for vitamin A by excluding all animal sources. The data reflect current vitamin A values from USDA Revised Handbook 8 (USDA, 1976–1987) updated by analytical data for tomatoes. Data do *not* represent total carotenoid content.

Survey (NHIS). The 1987 NHIS is a large, representative survey of the non-institutionalized U.S. adult population aged 19–99 years. The data in Table 2 reflect not only the concentration of vitamin A in individual foods but also their frequency of consumption and usual portion size. For example, liver, which is infrequently consumed by Americans, is the second leading source of vitamin A in the U.S. diet because of its high vitamin density. The primary sources of provitamin A carotenoids were derived from the vitamin A data in Table 2 by excluding animal sources of vitamin A. A limited number of vegetables—carrots, sweet potatoes, spinach, beef stew containing carrots, vegetable soup, collards/greens, broccoli, and green salad—provide nearly 92% of the provitamin A carotenoid content of the U.S. diet. The data also indicate that fruits in general are poor sources of beta-carotene. However, vegetables (and some fruits) which are high in carotenoids which are not converted to vitamin A, such as lycopene-rich tomatoes, do not appear on this list though they may be significant sources of total carotenoids. This table illustrates the difficulty in estimating carotenoid values from vitamin A data. It should be noted that carotenoids have also been found in small amounts in dairy products, eggs, shellfish, and poultry (Dimitrov, 1986).

ABSORPTION, TRANSPORT, AND LOCALIZATION

Dietary carotenoids are absorbed directly through the intestine; some are incorporated into chylomicra, appearing in the blood via the lymph (Bendich and Olson, 1989). The typical efficiency of absorption of carotenoids is assumed to be about one-third that of vitamin A (all-*trans*-retinol), based on the calculated absorption of beta-carotene. Research suggests that absorption of beta-carotene may be reduced by *in vivo* structural changes such as double-bond isomerization, ring opening, and oxidation (Beecher and Khachik, 1984). Carotenoids, which are fat-soluble, are transported in the blood primarily by low-density lipoproteins and, to a lesser extent, by high- and very-low-density lipoproteins (Parker, 1989). Most of the carotenoids distributed in human tissue are localized in adipose tissue (80–85%), followed by liver (8–12%) and muscle (2–3%). In addition, high concentrations of carotenoids are found in the corpus luteum and adrenal glands. Of the total body pool of carotenoids, serum contains approximately 1% (Bendich and Olson, 1989). Depending on an individual's vitamin A status, protein status, and metabolic characteristics, a significant amount of the provitamin A carotenoids are converted to retinal by the intestinal mucosa and to a lesser extent by the liver and other organs. Based on data for beta-carotene, the efficiency of conversion is assumed to be approximately 50%. The two possible pathways of conversion are central and eccentric cleavage. The retinal formed can then be reduced to retinol for use in vitamin A functions (Bendich and Olson, 1989).

CAROTENOIDS IN HUMANS

The individual carotenoids in humans at present. Five to 10 distinct carotenoids are found in the diet of U.S. subjects. Beta-carotene, alpha-carotene, and lycopene are the major components with lycopene being the most abundant (Parker, 1989). It is important to note that carotenoid levels in the blood are not highly correlated with relatively recent diet while serum carotenoid levels show a narrow range in well-nourished populations.

RELATIONSHIP BETWEEN DIETARY INTAKE AND BLOOD LEVELS

A recent study involving 30 men showed that individuals maintain relatively constant plasma concentrations of carotenoids which seemed to reflect relatively long-term dietary intakes of carotenoid-rich foods (Dimitrov et al., 1986). The maximum plasma concentrations of carotenoids from a single dose of pure beta-carotene or beta-carotene-rich foods were highly variable among subjects. A strong correlation was noted between the lowest and highest plasma concentrations which were consistently about 20% higher than the equivalent dose of beta-carotene intake of broccoli or tomato juice diet. Another feeding study of 61 men showed that interindividual variation in fasting plasma carotenoid levels to beta-carotene doses (Dimitrov et al., 1986) was significantly greater increases than those reported in the studies discussed above relied primarily on beta-carotene. This study showed that a test meal high in beta-carotene and moderate in vitamin A (40–50% of the average intake) and carotenoids (20% of the average intake) increased plasma carotenoids during the 4 hr feeding period suggests that subjects may not need to consume large amounts of beta-carotene to maintain high plasma values.

Several epidemiological studies have shown a relationship between dietary carotenoid intake, as measured by serum carotenoid levels ($r = 0.21-0.29$) (Dimitrov et al., 1985; Roidt et al., 1988). In addition, beta-carotene has been shown to affect plasma carotenoid levels in men. The plasma carotenoid levels were higher in men than in two studies (Wil-

CAROTENOIDS IN HUMAN SERUM

The individual carotenoids in human serum are also being measured by HPLC at present. Five to 10 distinct carotenoids have been identified in serum from U.S. subjects. Beta-carotene, alpha-carotene, cryptoxanthin, lycopene, and lutein are the major components with lycopene occurring at the highest concentrations (Parker, 1989). It is important to note that the levels of beta-carotene and retinol in the blood are not highly correlated. Serum beta-carotene is believed to reflect relatively recent diet while serum vitamin A appears to be maintained within a narrow range in well-nourished populations.

RELATIONSHIP BETWEEN DIETARY INTAKE AND BLOOD LEVELS

A recent study involving 30 men showed that for seven major plasma carotenoids, individuals maintain relatively constant carotenoid profiles. Fasting serum levels seemed to reflect relatively long-term dietary patterns and not occasional large intakes of carotenoid-rich foods (Brown et al., 1989a). The study showed that maximum plasma concentrations of beta-carotene occurred 24–48 hr after a dose of pure beta-carotene or beta-carotene in carrots, and that plasma responses were highly variable among subjects. A three- to fourfold difference in plasma levels was noted between the lowest and highest responders. However, concentrations were consistently about 20% higher when pure beta-carotene, rather than an equivalent dose of beta-carotene in carrots, was administered. An equivalent intake of broccoli or tomato juice did not change the levels of plasma carotenoids. Another feeding study of 61 men and women also indicated that there is wide interindividual variation in fasting plasma beta-carotene levels following beta-carotene doses (Dimitrov et al., 1988). Individuals on high-fat diets showed significantly greater increases than those on low-fat diets. While the two feeding studies discussed above relied primarily on fasting blood samples, another small study showed that a test meal high in calories (790 kcal) and fat (45% of calories), and moderate in vitamin A (40–50% of the Recommended Dietary Allowance) and carotenoids (20% of the average daily intake), did not alter seven major plasma carotenoids during the 4 hr after the meal (Brown et al., 1989b). This suggests that subjects may not need to be fasting to obtain useful blood carotenoid values.

Several epidemiological studies have shown limited associations between dietary carotenoid intake, as measured by food frequency questionnaires, and plasma carotenoid levels ($r = 0.21-0.29$) (Willett et al., 1983; Russell-Briefel et al., 1985; Roidt et al., 1988). In addition, factors other than dietary intake were shown to affect plasma carotenoid levels. Women exhibited significantly higher levels than men in two studies (Willett et al., 1983; Nierenberg et al., 1989).

While there is a possibility for bias when cancer patients recall usual dietary patterns, no clear evidence of bias exists in a number of well-conducted studies. However, selecting an appropriate time for blood collection so that nutrient levels will not be influenced by disease or treatment is often impossible in a retrospective study.

In this chapter the results of the epidemiological studies are evaluated by examining whether there is an association between carotenoids and cancer, its direction, and its statistical significance, and whether there is a graded response to increasing exposure and the statistical significance of the trend. Associations and trends can be biologically meaningful without being statistically significant; studies with small numbers may lack the power to attain statistical significance.

PROSPECTIVE STUDIES OF CAROTENOID INTAKE AND CANCER

Six prospective studies have examined the relationship between carotenoid intake and cancer (Hirayama, 1979, 1985; Shekelle et al., 1981; Kvale et al., 1983; Colditz et al., 1985; Wang and Hammond, 1985; Paganini-Hill et al., 1987) (Table 3). The earliest was published a little over 10 years ago (Hirayama, 1979). Three monitored cancer incidence (Shekelle et al., 1981; Kvale et al., 1983; Paganini-Hill et al., 1987) and three monitored cancer mortality (Hirayama, 1985; Colditz et al., 1985; Wang and Hammond, 1985). Only one study asked about most of the major carotenoid sources in the diet and formed a quantitative index of carotenoid intake using food composition tables (Paganini-Hill et al., 1987). A second study did develop an approximate carotenoid index but had to rely on summaries of diet histories rather than the original data (Shekelle et al., 1981). The other four studies used the frequency of consumption of a limited number of vegetables and fruits, not all of which were especially high in carotenoids (Kvale et al., 1983; Hirayama, 1985; Colditz et al., 1985; Wang and Hammond, 1985).

In three of the four studies that examined all cancers combined, risk was inversely related to vegetable and fruit or carotenoid intake (Hirayama, 1985; Colditz et al., 1985; Paganini-Hill et al., 1987). The two studies that analyzed men and women separately noted the inverse relationship with all cancer in both sexes (Hirayama, 1985; Paganini-Hill et al., 1987). Lung was the site most frequently involved. Decreased risk with increased intake was seen in four of the five studies that evaluated lung cancer (Shekelle et al., 1981; Kvale et al., 1983; Hirayama, 1985; Wang and Hammond, 1985), even though the single study that developed a quantitative measure of carotenoid intake found no association with lung cancer (Paganini-Hill et al., 1987). Cancer at several other sites [stomach (Hirayama, 1985), cervix (Hirayama, 1985), head and neck (Shekelle et al., 1981), breast (Paganini-Hill et al., 1987), and bladder (Paganini-Hill

Table 3 Prospective Studies of Dietary Carotenoids and Cancer

Authors, date	Study population	Exposure evaluated	Cancer site	No. of cases ^a	Evidence of association ^b
Hirayama, 1979, 1985	Japan Men, women	Green-yellow vegetables	All cancer	14,740	tr, neg (M,F)
			Stomach	5,247	tr, neg (M,F)
			Lung	1,917	tr, neg (M only)
			Cervix	589	+, neg
Shekelle, 1981	Western Electric Co. employees, Chicago, IL Men	Carotenoids in vegetables, fruits, and soups	All cancer	208	—
			Nonmelanoma skin	36	—
			Lung	33	+, tr, neg
			Prostate	29	—
			Colon	29	—
			Rectum	20	—
			Bladder	19	—
			Epidermoid head, neck	14	(+), neg
			Lung	70	(+), neg
			Kvale, 1983	Norway Men	Vegetables (excl. potatoes) Fruits, berries Six vegetables and fruits
Colditz, 1985	Elderly MA residents Men, women				
Wang, 1985	Volunteers from 25 U.S. states (American Cancer Society cohort) Men	Fruits and fruit juices Green salad	Lung	671	tr(?), neg
Paganini-Hill, 1987	Residents of CA retirement community (Leisure World) Men, women	Carotenoids	All cancer	638	(tr), neg (M) + (?), neg (F) (+), neg (F)
			Breast	123	—
			Colon	110	—
			Prostate	92	—

Wang, 1985	Volunteers from 25 U.S. states (American Cancer Society cohort) Men	Fruits and fruit juices Green salad	Lung	671	tr(?), neg tr(?), neg
Paganini-Hill, 1987	Residents of CA retirement community (Leisure World) Men, women	Carotenoids	All cancer Breast Colon Prostate Bladder Lung	638 123 110 92 58 55	(tr), neg (M) + (?), neg (F) (+), neg (F) — — (tr), neg (M) tr, neg (F) —

^a Refers to the number of cancer cases used to evaluate a relationship with carotenoids. This number may be less than the cancer incidence or mortality within the cohort because of missing information on diet and potential confounders.

^b A + indicates a statistically significant association, e.g., a significant difference in dietary intake between cases and noncases/controls or a significant difference in cancer rates between subgroups of the cohort stratified by dietary intake; (-) indicates an apparent association that is not statistically significant; + (?) indicates an apparent association that was not tested for statistical significance. Tr indicates a statistically significant test for trend in cancer rates or rate ratios with changes in dietary intake; (tr) indicates an apparent trend that is not statistically significant; neg implies a decreased risk of cancer with increased intake.

et al., 1987)] was reported to be reduced with increased intake of vegetables and fruits or carotenoids in a single study. However, two studies failed to find a protective effect for colon cancer (Shekelle et al., 1981; Paganini-Hill et al., 1987); two failed to find one for prostate cancer (Shekelle et al., 1981; Paganini-Hill et al., 1987); and one of two studies failed to find a protective effect for bladder cancer (Shekelle et al., 1981; Paganini-Hill et al., 1987). Only two of these studies systematically evaluated cancer at different sites (Shekelle et al., 1981; Paganini-Hill et al., 1987). In addition, the less common cancers would not yet have occurred in sufficient numbers in these cohorts to be analyzed.

Taken together, these six prospective studies of diet and cancer strongly suggest that high levels of vegetable and fruit consumption are associated with a reduced risk of lung cancer and possibly other cancers. The causal agent is more difficult to identify. Only one study systematically investigated the relationship of all the major nutrients to risk of lung cancer (Shekelle et al., 1981). Carotenoid intake alone was significantly associated with reduced risk. Vitamin C, which like carotenoids is found primarily in vegetables and fruits, was not implicated in the two studies that evaluated it (Shekelle et al., 1981; Kvale et al., 1983). No decrease in risk of lung cancer with high retinol (preformed vitamin A) consumption was noted in two studies (Shekelle et al., 1981; Paganini-Hill et al., 1987) although results of a third study did suggest such a relationship (Kvale et al., 1983). Finding a reduction in risk with consumption of carotenoids but not retinol suggests that the active carotenoids do not first have to be metabolized into vitamin A to be protective.

The maximum follow-up time in these prospective studies ranged from 5 years (Colditz et al., 1985; Paganini-Hill et al., 1987) to 11–12 years (Kvale et al., 1983; Wang and Hammond, 1985) to 17–19 years (Shekelle et al., 1981; Hirayama, 1985). In the longer duration studies, it seems likely that current diet assessed at onset of follow-up did not reflect preclinical disease. One of the studies with extended follow-up did examine the influence of time between dietary interview and diagnosis of lung cancer on the strength of the carotenoid association and found no effect (Shekelle et al., 1981).

In general, these studies did not demonstrate in their published reports that smoking was adequately controlled. Intake of vegetables and fruits and carotenoids is decreased among smokers (Stryker et al., 1988; Subar et al., 1990). In a recent study the carotenoid intake of male smokers was reported to be 80–90% that of male nonsmokers; the comparable range for females was 70–80% (Stryker et al., 1988). Thus uncontrolled confounding by smoking might generate an apparent protective effect for diet in studies of lung cancer and other smoking-related cancers, such as head and neck, bladder, and cervix. Most of the studies adjusted for smoking intensity. Only one study adjusted for duration of smoking (Shekelle et al., 1981), which is a stronger predictor of lung cancer risk than intensity

(Doll and Peto, 1978). Also, some to control for confounding because

PROSPECTIVE STUDIES OF AND CANCER

Prospective epidemiological studies and carotenoid intake but also carot onset of cancer. These six studies a Stahelin et al., 1984, 1991; Nomura et al., 1987; Wald et al., 1988b; B Connett et al., 1989). In the 5 ye published, the approach has become enoids were assayed spectrophotom was used to separate and measure be individual carotenoids, such as lyco (Burney et al., 1989; Helzlsouer et

In five of these prospective studi developed cancer were selected from stored blood samples would need to al., 1985; Menkes et al., 1986; Schol et al., 1989; Helzlsouer et al., 198 measured beta-carotene in each partic lected (Stahelin et al., 1991). Four o et al., 1984; Nomura et al., 1985; M Wald et al., 1988b; Burney et al., 1 mortality (Stahelin et al., 1991; Con

All of the studies systematically test cancers in their populations. In all five of lung cancer was reduced among su (Nomura et al., 1985; Menkes et al., 1989; Stahelin et al., 1991). The inver in four of these studies (Nomura et al., 1988b; Stahelin et al., 1991) and trend carotenoid levels were not related to al., 1984), in another study total carot verse association with lung cancer than In all three studies that examined sto beta-carotene levels (Nomura et al., 1991) although the relationship was For colon cancer inverse associati

(Doll and Peto, 1978). Also, some of these studies were limited in their ability to control for confounding because of small numbers of cancers.

PROSPECTIVE STUDIES OF BLOOD CAROTENOID LEVELS AND CANCER

Prospective epidemiological studies have considered not only vegetable and fruit and carotenoid intake but also carotenoid levels in serum or plasma prior to the onset of cancer. These six studies are presented in Table 4 (Willett et al., 1984; Stahelin et al., 1984, 1991; Nomura et al., 1985; Menkes et al., 1986; Schober et al., 1987; Wald et al., 1988b; Burney et al., 1989; Helzlsouer et al., 1989; Connett et al., 1989). In the 5 years since the earliest of these studies was published, the approach has become more sophisticated. Originally total carotenoids were assayed spectrophotometrically (Willett et al., 1984); then HPLC was used to separate and measure beta-carotene. Recently, blood levels of other individual carotenoids, such as lycopene and lutein, have also been determined (Burney et al., 1989; Helzlsouer et al., 1989; Comstock et al., 1991).

In five of these prospective studies controls matched to the individuals who developed cancer were selected from the cohort so that a limited number of the stored blood samples would need to be thawed (Willett et al., 1984; Nomura et al., 1985; Menkes et al., 1986; Schober et al., 1987; Wald et al., 1988b; Burney et al., 1989; Helzlsouer et al., 1989; Connett et al., 1989). The sixth study measured beta-carotene in each participant's blood immediately after it was collected (Stahelin et al., 1991). Four of the studies used cancer incidence (Willett et al., 1984; Nomura et al., 1985; Menkes et al., 1986; Schober et al., 1987; Wald et al., 1988b; Burney et al., 1989; Helzlsouer et al., 1989); two, cancer mortality (Stahelin et al., 1991; Connett et al., 1989).

All of the studies systematically tested for associations with each of the common cancers in their populations. In all five studies that measured beta-carotene, risk of lung cancer was reduced among subjects with high blood beta-carotene levels (Nomura et al., 1985; Menkes et al., 1986; Wald et al., 1988b; Connett et al., 1989; Stahelin et al., 1991). The inverse associations were statistically significant in four of these studies (Nomura et al., 1985; Menkes et al., 1986; Wald et al., 1988b; Stahelin et al., 1991) and trends were apparent in all five. Although total carotenoid levels were not related to lung cancer risk in one study (Willett et al., 1984), in another study total carotenoid levels demonstrated a stronger inverse association with lung cancer than beta-carotene levels (Connett et al., 1989). In all three studies that examined stomach cancer, risk was reduced with high beta-carotene levels (Nomura et al., 1985; Wald et al., 1988b; Stahelin et al., 1991) although the relationship was not as pronounced as for lung cancer. For colon cancer inverse associations with beta-carotene were observed

Table 4 Prospective Studies of Serum or Plasma Carotenoids and Cancer

Authors, date	Study population	Exposure evaluated	Cancer site	No. of cases, controls ^a	Evidence of association ^b
Willett, 1984	14 centers in U.S. hypertension study (HDFFP) Men, women	Total carotenoids	All cancer Lung Breast Leukemia, lymphoma Gastrointestinal Prostate Lung Stomach Colon Other cancers	111,210 17,28 14,31 11,23 11,22 11,21 68 20 17 99	— — — +, pos — — +, tr(?), neg +, neg — —
Stahelin, 1984, 1991	Chemical co. employees, Basel, Sw Men	Beta-carotene	Colon Lung Stomach Rectum Bladder Lung Colon	81,302 74,302 70,302 32,302 27,302 99,196 72,143	(+), neg +, tr, neg (+), neg — — +, tr, neg —
Nomura, 1985	Japanese in heart disease study, Oahu, HI Men	Beta-carotene	Pancreas	22,44	—
Menkes, 1986 Schober, 1987 Burney, 1989	Washington County, MD Men, women	Beta-carotene Beta-carotene	Bladder	35,70	+, tr, neg — (+), (tr), neg
Helzlsouer, 1989		Total carotenoids Beta-carotene Lycopene Beta-carotene Lycopene			
Wald, 1988b	Users of BUPA medical center, London, UK Men	Beta-carotene	All cancer Skin Lung Colorectal Central nervous system Bladder Stomach Other	271,533 56,107 50,99 30,59 17,34	+, tr, neg — +, tr, neg (+), neg (+), neg (+), neg (+), neg —
Connett, 1989	22 centers in U.S. heart disease study (MRFIT) Men	Total carotenoids Beta-carotene Total carotenoids Beta-carotene Total carotenoids	All cancer Lung cancer Gastrointestinal	156,311 66,131 28,56	— +, tr, neg (+), (tr), neg — —

Wald, 1988b	Users of BUPA medical center, London, UK Men	Beta-carotene	All cancer	271,533	+ , tr, neg
			Skin	56,107	—
			Lung	50,99	+ , tr, neg
			Colorectal	30,59	(+), neg
			Central nervous system	17,34	(+), neg
			Bladder	15,29	(+), neg
			Stomach	13,26	+ (?), neg
			Other	90,179	—
Connert, 1989	22 centers in U.S. heart disease study (MRFIT) Men	Total carotenoids Beta-carotene	All cancer	156,311	—
		Total carotenoids Beta-carotene	Lung cancer	66,131	+ , tr, neg
		Total carotenoids Beta-carotene	Gastrointestinal	28,56	(+), (tr), neg
		Total carotenoids Beta-carotene	Colon	14,28	—
					—

^a Refers to the number of cases and matched controls selected from the cohort that were used to evaluate a relationship with blood carotenoid levels or to the number of cases alone if the entire cohort was utilized in analysis.

^b A + indicates a statistically significant association, e.g., a significant difference in blood nutrient levels between cases and controls or a significant difference in relative risks between subgroups of the study population stratified by blood nutrient levels; (+) indicates an apparent association that is not statistically significant; + (?) indicates an apparent association that was not tested for statistical significance. Tr indicates a statistically significant trend in relative risks with changes in blood nutrient levels; (tr) indicates an apparent trend that is not statistically significant; tr(?) indicates an apparent trend that was not tested for statistical significance. Pos implies an increased risk of cancer with increased nutrient levels; neg implies a decreased risk of cancer with increased nutrient levels.

in only two of five investigations (Nomura et al., 1985; Wald et al., 1988b); for bladder cancer, in one of three investigations (Wald et al., 1988b).

The apparent reduction in risk of pancreas and bladder cancer with high serum lycopene levels (Burney et al., 1989; Helzlsouer et al., 1989) is provocative but needs to be replicated by further research. The absence of parallel effects for beta-carotene suggests that high lycopene levels measure something more specific than frequent consumption of vegetables and fruits and that not all antioxidant carotenoids may be equally effective *in vivo*.

In general, the specificity of the beta-carotene associations noted in these prospective studies of blood nutrient levels and cancer has not been adequately explored. Blood levels of other carotenoids and other constituents of vegetables and fruits, such as vitamin C and folacin, have not been systematically evaluated. For example, only the study that assayed for micronutrients immediately after blood collection was able to measure vitamin C because of its lability in stored serum and plasma. Vitamin C was significantly reduced in those men who subsequently developed stomach cancer but unaltered in those who developed lung cancer (Stahelin et al., 1991). Techniques are now available to stabilize the vitamin C in blood for long-term storage (Margolis et al., 1990) and need to be considered in future cohort studies. Vitamins A and E were the only nutrients routinely assayed along with beta-carotene. Among the six studies strong or consistent associations for vitamin A similar to those seen for beta-carotene were not seen, suggesting that beta-carotene need not first be converted to vitamin A to be active. Inverse associations with blood vitamin E levels were noted in three of the six studies (Willett et al., 1984; Stahelin et al., 1984; Menkes et al., 1986) but may have resulted from inverse associations of blood lipoprotein levels with risk of cancer.

Except for one study with five years of follow-up (Willett et al., 1984), the maximum follow-up time in these cohorts ranged from 8 to 12 years. However, median times between blood collection and cancer diagnosis or death are likely to be much shorter and, in general, were not presented. Among the five studies that examined the impact of elapsed time between blood collection and lung cancer (Stahelin et al., 1984; Nomura et al., 1985; Menkes et al., 1986; Wald et al., 1988b; Connett et al., 1989), four were able to demonstrate that lowered blood beta-carotene levels in the subjects who eventually developed lung cancer were not likely to be a result of preclinical disease (Stahelin et al., 1984; Nomura et al., 1985; Menkes et al., 1986; Wald et al., 1988b) even though data from three studies suggest that beta-carotene levels are reduced in the years immediately preceding lung cancer diagnosis (Wald et al., 1988b) or death (Stahelin et al., 1984; Connett et al., 1989). It is interesting to note that in the London BUPA cohort serum levels of beta-carotene, vitamin A, and vitamin E were all reduced in those men who subsequently developed lung cancer but the low levels of vitamin A and vitamin E were restricted to those men who were diagnosed within

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a few years after blood collection ; quences of cancer (Wald et al., 19 levels were thought to be etiologic;

Degradation of carotenoids duri worrisome problem. At present the under different storage conditions is the U.S. National Institute for Stan for Disease Control, are conductir problem is illustrated by one cohort undetectable levels of beta-carotene years (Friedman et al., 1986). In a to -70°C , 15% of the specimens 1 years (Connett et al., 1989). A th approximately 5% per year in beta-ca up to 10 years (Wald et al., 1988b). totally reliable (Nomura et al, 198 carotenoids leads to a less precise n a study to detect associations is dimir possible, most of these prospective of storage (Willett et al., 1984; Men et al., 1988b; Connett et al., 1989) analysis (Willett et al., 1984).

A recent study of carotenoid sta immediately after plasma separation room temperature in the dark for carotenoids (lutein/zeanthin, precryl carotene, and beta-carotene) were sta or at -20°C for 5 months. Howev at -20°C were significantly decreas data based on blood samples stored t -70°C may be questionable.

Of more concern than the ability tionships is its potential to generate : occur if the carotenoids in the blood at different rates. This situation pro ducted in Guernsey, England (Wald were lower among the 39 women w follow-up than among the 78 match further investigation indicated that b storage at -20°C and was likely to samples from the cases because of r (1988a).

a few years after blood collection and thus were attributed to metabolic consequences of cancer (Wald et al., 1986, 1987). Only the reduced beta-carotene levels were thought to be etiologically important (Wald et al., 1988b).

Degradation of carotenoids during extended storage of blood samples is a worrisome problem. At present the precise stability of individual carotenoids under different storage conditions is not known although several groups, including the U.S. National Institute for Standards and Technology and the U.S. Centers for Disease Control, are conducting relevant experiments. The scope of the problem is illustrated by one cohort study where 83% of the serum samples had undetectable levels of beta-carotene after storage at -23° to -40°C for 12–19 years (Friedman et al., 1986). In another study which stored samples at -50° to -70°C , 15% of the specimens had no beta-carotene ($<2\ \mu\text{g}/\text{dl}$) after 8–10 years (Connett et al., 1989). A third study reported a gradual decline of approximately 5% per year in beta-carotene levels in serum stored at -40°C for up to 10 years (Wald et al., 1988b). Not even storage at -70°C has been proved totally reliable (Nomura et al., 1985; Menkes et al., 1986). If degradation of carotenoids leads to a less precise measurement of exposure, then the power of a study to detect associations is diminished. However, to compensate to the extent possible, most of these prospective studies matched controls to cases by length of storage (Willett et al., 1984; Menkes et al., 1986; Schober et al., 1987; Wald et al., 1988b; Connett et al., 1989) and/or standardized for storage interval in analysis (Willett et al., 1984).

A recent study of carotenoid stability indicates that plasma samples frozen immediately after plasma separation were no different from those maintained at room temperature in the dark for 24 hr (Craft et al., 1988). Six individual carotenoids (lutein/zeaxanthin, precryptoxanthin, cryptoxanthin, lycopene, alpha-carotene, and beta-carotene) were stable in plasma stored at -70°C for 28 months or at -20°C for 5 months. However, after 15 months carotenoids maintained at -20°C were significantly decreased. Such results suggest that the validity of data based on blood samples stored for long periods at temperatures greater than -70°C may be questionable.

Of more concern than the ability of carotenoid degradation to obscure relationships is its potential to generate associations that do not exist. Such bias can occur if the carotenoids in the blood collected from cases and controls degrade at different rates. This situation probably occurred in a prospective study conducted in Guernsey, England (Wald et al., 1984). Plasma beta-carotene levels were lower among the 39 women who developed cancer during 7–14 years of follow-up than among the 78 matched controls, and a trend was apparent. But further investigation indicated that beta-carotene had degraded markedly during storage at -20°C and was likely to have been more rapidly destroyed in blood samples from the cases because of repeated freezing and thawing (Wald et al., 1988a).

In these prospective studies of blood carotenoid levels and cancer, as in the studies of dietary carotenoids, control of smoking is critical because plasma beta-carotene and total carotenoids have been shown to be reduced among smokers relative to nonsmokers (Russell-Briefel et al., 1985; Stryker et al., 1988; Nierenberg et al., 1989), and plasma beta-carotene is inversely correlated with frequency of smoking (Stryker et al., 1988). One reason is that smokers consume fewer vegetables, fruits, and carotenoids; but in addition, beta-carotene levels may rise less sharply with increasing carotenoid intake in smokers. In a recent study, men and women who smoked one pack a day had on the average 72 and 79%, respectively, of the plasma beta-carotene levels of nonsmokers with similar carotenoid intake (Stryker et al., 1988). The possibility exists that beta-carotene in the blood may quench free radicals in cigarette smoke. Thus adjusting beta-carotene effects for smoking may lead to an underestimate of the protective effect of beta-carotene. Nonetheless, control of smoking is still necessary for a conservative estimate of the relationship between blood carotenoid levels and risk of any smoking-related cancer.

Dietary intake and blood nutrient levels are imperfect but complementary ways of evaluating carotenoids. High dietary intake of carotenoids may simply reflect high intake of vegetables and fruits and their constituents, such as vitamin C, folate, dietary fiber, and indoles. High levels of beta-carotene in serum or plasma may reflect high dietary intake of carotenoids, vegetables, and fruits or other dietary and genetic factors that influence the low-density lipoprotein fraction which transports beta-carotene in blood. The concordance of the studies of dietary carotenoids and blood beta-carotene levels—both measures are associated with a reduced risk of lung cancer—suggests that beta-carotene may be the protective factor. This is the simplest explanation although a role for other carotenoids or other constituents of vegetables and fruits is consistent with the findings.

In comparing prospective studies of the influence of dietary carotenoids and blood beta-carotene levels, the latter are often considered more valid because a well-characterized chemical species is being measured. However, conclusions based on the former have more immediate public health relevance since recommendations about reducing cancer risk must be expressed in practical terms of dietary intake.

RETROSPECTIVE STUDIES OF CAROTENOID INTAKE AND LUNG CANCER

In addition to the prospective studies reviewed, a number of retrospective studies of carotenoids and specific cancers have been conducted. Lung has been the site studied most intensively because of the prevalence of this cancer and the increasing evidence from epidemiological studies that diet may be involved in its etiology. The 11 retrospective studies of carotenoid intake and lung cancer are summarized

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in Table 5 (MacLennan et al., 1986; Samet et al., 1985; Wu et al., 1987; Bond et al., 1987; Pastorino et al., 1989). Retrospective studies of diet and lung cancer were not included. The severity of this cancer is such that appetite and metabolism would be expected to influence interpretation of blood nutrient levels.

All 11 studies of diet and lung cancer used either the intake of carotenoids and/or vegetables and fruits. In nine of the studies indices of carotenoid intake were used. In the remaining two studies (Ziegler et al., 1984, 1986; Samet et al., 1987; Bond et al., 1987; Pastorino et al., 1989) by including vegetables and fruits in the dietary interview, and adjusting for total energy according to carotenoid content from the diet.

Two of these studies attempted to adjust for total energy intake of carotenoids or another constituent which may be responsible for the protective effect. In the first study (Ziegler et al., 1984) of dark green and dark yellow-orange vegetables, the index of intake index, and more pronounced decrease in lung cancer risk for the two food group measures (Ziegler et al., 1984) presented: (1) beta-carotene was preferred to total beta-carotene, were better measures of intake of the hydrocarbon carotenoids; or (2) total carotenoids or another constituent of these vegetables. In another study total vegetable intake was associated with a lower risk of lung cancer than estimates of total carotenoids with vitamin A activity (Ziegler et al., 1984) which are rich in lycopene; dark green vegetables, rich in indoles, seemed to be a better measure in beta-carotene. These observations suggest that other than beta-carotene might be responsible for the protective effect. Further research regarding individual carotenoids and estimation of the intake of the specific carotenoids to identify the actual protective factor is needed.

In general, in these retrospective studies, the intake of carotenoids concentrated in vegetables and fruits was associated with a lower risk of lung cancer. In the remaining two studies (Byers et al., 1987; Pastorino et al., 1989) but one (Fontham et al., 1988) the as

in Table 5 (MacLennan et al., 1977; Hinds et al., 1984; Ziegler et al., 1984, 1986; Samet et al., 1985; Wu et al., 1985; Pisani et al., 1986; Byers et al., 1987; Bond et al., 1987; Pastorino et al., 1987; Fontham et al., 1988; Marchand et al., 1989). Retrospective studies of blood carotenoid levels and lung cancer are not included. The severity of this particular cancer and its treatment suggests that appetite and metabolism would be altered after diagnosis and complicate the interpretation of blood nutrient levels.

All 11 studies of diet and lung cancer showed decreased risk with increased intake of carotenoids and/or vegetable and fruit subgroups. In all the studies either the inverse associations or the tests for trend were statistically significant. In nine of the studies indices of carotenoid intake were developed (Hinds et al., 1984; Ziegler et al., 1984, 1986; Samet et al., 1985; Wu et al., 1985; Byers et al., 1987; Bond et al., 1987; Pastorino et al., 1987; Fontham et al., 1988; Marchand et al., 1989) by including most of the carotenoid-rich vegetables and fruits in the dietary interview, and weighting the frequencies of consumption according to carotenoid content from food composition tables.

Two of these studies attempted to determine whether beta-carotene or other carotenoids or another constituent of vegetables and fruits was primarily responsible for the protective effect. In one study the frequencies of consumption of dark green and dark yellow-orange vegetables were compared with a carotenoid index, and more pronounced decreases in lung cancer risk were associated with the two food group measures (Ziegler et al., 1986). Two explanations were presented: (1) beta-carotene was protective; and these two food groups, rich in beta-carotene, were better measures of its intake than an approximate index of the hydrocarbon carotenoids; or alternatively (2) the protective agent might be another constituent of these vegetable subgroups, and not necessarily a carotenoid. In another study total vegetable intake was more strongly associated with reduced risk of lung cancer than estimates of the intake of beta-carotene or of the other carotenoids with vitamin A activity (Marchand et al., 1989). In addition, tomatoes, which are rich in lycopene; dark green vegetables, rich in lutein; and cruciferous vegetables, rich in indoles, seemed about as protective as carrots, which are rich in beta-carotene. These observations suggested that constituents of vegetables other than beta-carotene might be important in the prevention of lung cancer. Further research regarding individual carotenoid content of foods will enable estimation of the intake of the specific carotenoids and will thus facilitate studies to identify the actual protective factor(s).

In general, in these retrospective studies of diet and lung cancer other factors concentrated in vegetables and fruits have been rarely investigated. Inverse associations with vitamin C were noted in four studies (Hinds et al., 1984; Byers et al., 1987; Fontham et al., 1988; Marchand et al., 1989) and with dietary fiber in two studies (Byers et al., 1987; Marchand et al., 1989); but in every situation but one (Fontham et al., 1988) the associations were weaker than with carotenoids.

Table 5 Retrospective Studies of Dietary Carotenoids and Lung Cancer

Authors, date	Study population	Exposure evaluated	Cases, controls Type of controls	Evidence of association ^a
MacLennan, 1977	Singapore Chinese Men, women	8 vegetables (6 green leafy vegs.)	233,300 Hospital	+, neg
Hinds, 1984	Oahu, HI Multiethnic Men, women	Carotenoids	M: 261, 444 F: 103, 183 Population	+, tr(?), neg tr(?), pos
Ziegler, 1984, 1986	New Jersey Men	Carotenoids Dark green vegs. Dark yellow-orange vegs.	763, 900 Population	(tr), neg tr, neg tr, neg
Samet, 1985	New Mexico Men, women	Carotenoids	447, 759 Population	+, (tr), neg
Wu, 1985	Los Angeles, CA Women	Carotenoids	220, 440 Neighborhood	+, tr(?), neg
Pisani, 1986	Lombardy, Italy Men, women	Carrots Leafy green vegetables	417, 849 Hospital	tr, neg tr, neg
Byers, 1987	Upstate New York Men, women	Carotenoids	M: 296, 587 F: 154, 315 Neighborhood	+, tr, neg —
Bond, 1987	Chemical co. employees, Texas Men	Carotenoids	734 ^b Cohort ^c	+, tr(?), neg
Pastorino, 1987	Milan, Italy Women	Carotenoids	47, 159 Hospital	+, neg
Fonham, 1988	Southern Louisiana Men, women	Carotenoids Vegetables Fruits	1253, 1274 Hospital	(+), (tr), neg (+), (tr), neg +, tr, neg
Marchand, 1989	Oahu, HI Multiethnic Men, women	Beta-carotene Other provitamin A carotenoids Vegetables Fruits Beta-carotene	M: 230, 597 W: 102, 268	+, tr, neg + (?), tr, neg +, tr, neg — +, tr, neg

Pastorino, 1987	Milan, Italy Women	47, 159 Hospital	Carotenoids	+ , neg
Fontham, 1988	Southern Louisiana Men, women	1253, 1274 Hospital	Carotenoids Vegetables Fruits	(+), (tr), neg (+), (tr), neg + , tr, neg
Marchand, 1989	Oahu, HI Multiethnic Men, women	M: 230, 597	Beta-carotene Other provitamin A carotenoids Vegetables Fruits	+ , tr, neg + , tr, neg + (?), tr, neg + , tr, neg
		W: 102, 268 Population	Beta-carotene Other provitamin A carotenoids Vegetables Fruits	— + , tr, neg + (?), tr, neg + , tr, neg

^a A + indicates a statistically significant association, e.g., a significant difference in dietary intake between cases and controls or a significant difference in relative risks between subgroups of the study population stratified by dietary intake; (+) indicates an apparent association that is not statistically significant; + (?) indicates an apparent association that was not tested for statistical significance. Tr indicates a statistically significant trend in relative risks with changes in dietary intake; (tr) indicates an apparent trend that is not statistically significant; tr(?) indicates an apparent trend that was not tested for statistical significance. Pos implies an increased risk of cancer with increased intake; neg implies a decreased risk of cancer with increased intake.

^b Represents the combined number of cases and controls participating in the study. Separate numbers of cases and controls were not given.

^c Controls matched to the cases were selected from the cohort.

Retinol intake was considered in nine studies (Hinds et al., 1984; Ziegler et al., 1984, 1986; Samet et al., 1985; Wu et al., 1985; Bond et al., 1987; Byers et al., 1987; Pastorino et al., 1987; Fontham et al., 1988; Marchand et al., 1989) and seemed unrelated to lung cancer risk in all but one (Fontham et al., 1988).

In many of these retrospective studies, as in the prospective studies described earlier, it is not clear that the carotenoid-lung cancer associations were adequately adjusted for smoking. Emphasis was placed on adjusting for smoking intensity although duration of smoking may have been a more potent confounder.

Six of the studies used general population (Hinds et al., 1984; Ziegler et al., 1984, 1986; Samet et al., 1985; Marchand et al., 1989) or neighborhood (Wu et al., 1985; Byers et al., 1987) controls rather than hospital controls. This approach eliminates the possibility that the dietary patterns that predispose toward many chronic diseases, such as heart disease, stroke, and diabetes, and characterize many hospitalized individuals of the same age as cancer patients may generate the dietary differences between cancer cases and controls observed in a study.

Even though all 11 of these retrospective studies indicate that intake of vegetables, fruits, and carotenoids may significantly reduce the risk of lung cancer, the more detailed findings are not as consistent. All seven of the studies conducted in men detected a decreased risk of lung cancer with high carotenoid intake (Hinds et al., 1984; Ziegler et al., 1984, 1986; Samet et al., 1985; Byers et al., 1987; Bond et al., 1987; Fontham et al., 1988; Marchand et al., 1989). Four studies observed the same relationship in women (Samet et al., 1985; Wu et al., 1985; Pastorino et al., 1987; Marchand et al., 1989); three did not (Hinds et al., 1983; Byers et al., 1987; Fontham et al., 1988). One study included whites and Hispanics and found the protective effect of dietary carotenoids restricted to whites (Samet et al., 1985), while another study with both white and black subjects found protection by dietary carotenoids in both races (Fontham et al., 1988). Two studies reported that protection was primarily among smokers who had stopped smoking (Samet et al., 1985; Byers et al., 1987), but a third found it predominantly among current smokers (Ziegler et al., 1986). A fourth study noted protective effects in both current and ex-smokers (Fontham et al., 1988); a fifth reported different patterns for men and women (Marchand et al., 1989).

One consistent finding of these case-control studies is that protection by carotenoids is not restricted to squamous cell lung cancer. Of the six studies that investigated histological specificity, all found inverse associations of carotenoid intake with lung adenocarcinoma (Wu et al., 1985; Pisani et al., 1986) or small-cell lung cancer (Fontham et al., 1988) or both (Ziegler et al., 1984; Byers et al., 1987; Marchand et al., 1989), although the associations seemed strongest with squamous cell disease. This result suggests that carotenoids might modulate carcinogenesis in sites other than just those involving squamous epithelium.

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Vitamin A plays an important role in the maintenance of the respiratory epithelium. Failure to observe a protective effect of carotenoids weakens the argument (De Vet et al., 1989) for a mechanism involving cleavage of carotenoids to retinol.

When the six studies that used neighborhood controls are considered, among those in the lowest quartile of intake, the risk of lung cancer in the highest quartile or tertile (Ziegler et al., 1984, 1986; Samet et al., 1985; Pastorino et al., 1987; Marchand et al., 1989). Not surprisingly, low levels of vegetable and fruit consumption in the community may be sufficient for risk. What needs to be more carefully examined is the care over which an effect is observed. In the studies on fruits, vegetables, and carotenoids seem to be associated with nutritionally inadequate diet that normally consumed might be

RETROSPECTIVE STUDIES AND OTHER CANCERS

For cancers other than lung cancer, case-control studies have been conducted or the results of prospective studies on the role of carotenoids. Those cancers for which case-control studies that vegetables, fruits, and carotenoids reduce the risk of mouth, pharynx, larynx, esophagus, and stomach cancer.

It needs to be emphasized that the protective effect of high vegetable and fruit intake on lung cancer etiology is the same as that observed in a population-based case-control study in Washington, D.C., the U.S. metropolitan area. In that study, mortality rates for nonwhite male were the dominant risk factor. However, low vegetable and fruit consumption was a risk factor for risk of esophageal cancer. Also, low intake of dairy product and egg consumption and high intake of fat (Table 6). Low intake of vitamin A and carotenoids, were also all associated with lung cancer that generally poor nutrition, characterized by low vegetable groups, was the dominant dietary risk factor that might be involved. This explanation

Vitamin A plays an important role in the normal differentiation of squamous epithelium. Failure to observe a similar histological specificity for carotenoids weakens the argument (De Vet, 1989) that beta-carotene functions through a mechanism involving cleavage into retinol in peripheral tissues.

When the six studies that used a carotenoid index and population or neighborhood controls are considered, the smoking-adjusted relative risks of lung cancer among those in the lowest quartile or tertile of carotenoid intake, compared to those in the highest quartile or tertile, ranged from 1.3 to 2.7 (Hinds et al., 1984; Ziegler et al., 1984, 1986; Samet et al., 1985; Wu et al., 1985; Byers et al., 1987; Marchand et al., 1989). Nonetheless, these relative risks suggest that the levels of vegetable and fruit consumption characteristic of about 30% of a typical community may be sufficient for a noticeable reduction (22-63%) in lung cancer risk. What needs to be more carefully examined is the range of carotenoid intake over which an effect is observed. It is important to determine whether vegetables, fruits, and carotenoids seem to reduce lung cancer risk only among individuals with nutritionally inadequate diets or whether increased carotenoid intake beyond that normally consumed might benefit well-nourished individuals.

RETROSPECTIVE STUDIES OF CAROTENOID INTAKE AND OTHER CANCERS

For cancers other than lung cancer, too few retrospective studies of the role of diet have been conducted or the results are too inconsistent to definitely implicate carotenoids. Those cancers for which there is suggestive evidence from retrospective studies that vegetables, fruits, and carotenoids are protective include mouth, pharynx, larynx, esophagus, stomach, colon, rectum, bladder, and cervix.

It needs to be emphasized that for these other sites evidence of reduced risk with high vegetable and fruit intake does not necessarily imply that the dietary etiology is the same as that observed with lung cancer. For example, we conducted a population-based case-control study of esophageal cancer among black men in Washington, D.C., the U.S. metropolitan area with the highest esophageal cancer mortality rates for nonwhite males (Ziegler et al., 1981). Heavy alcohol intake was the dominant risk factor. However, even after controlling for alcohol, low vegetable and fruit consumption was significantly associated with an increased risk of esophageal cancer. Also associated with increased risk were low dairy product and egg consumption and low fresh or frozen meat and fish consumption (Table 6). Low intake of vitamin C, riboflavin, and vitamin A, as well as of carotenoids, were also all associated with elevated risk. These results suggested that generally poor nutrition, characterized by inadequate intake of the basic food groups, was the dominant dietary risk factor. Multiple micronutrient deficiencies might be involved. This explanation is consistent with the geographic pattern of

Table 6 Adjusted^a Relative Risks of Esophageal Cancer in Washington, D.C. Black Males by Food Group and Micronutrient Intake^b

Food group or micronutrient	Level of consumption			<i>p</i> for trend
	Highest tertile	Middle tertile	Lowest tertile	
Meat and fish	1.0	1.3	1.2	0.39
Fresh or frozen meat and fish	1.0	1.6	2.2	0.01
Processed meat and fish	1.0	0.9	0.9	0.34
Dairy products and eggs	1.0	1.7	1.9	0.02
Vegetables and fruits	1.0	1.7	2.0	0.02
Vegetables	1.0	1.5	1.6	0.07
Fruits	1.0	2.4	2.0	0.05
Complex carbohydrates	1.0	1.1	1.2	0.24
Vitamin A	1.0	1.5	1.5	0.10
Carotenoids	1.0	1.3	1.3	0.17
Vitamin C	1.0	1.2	1.8	0.03
Thiamin	1.0	1.2	1.2	0.34
Riboflavin	1.0	1.0	1.7	0.05

^a Adjusted for ethanol consumption. Smoking was not a risk factor in this study.

^b Includes 120 cases and 250 controls.

Source: Adapted from Ziegler et al., 1981.

this cancer. Internationally it is endemic in regions with limited diets and impoverished agriculture; within a country it seems associated with low socioeconomic status.

Cervical cancer is another cancer that has been linked with carotenoids, vegetables, and fruits. It is primarily a squamous cell epithelial tumor like lung cancer. It is associated with low socioeconomic status and thus possibly with poor nutrition. Several epidemiological studies have suggested that carotenoids, vitamin C, or folacin might be protective (Ziegler et al., 1990). However, in a recent community-based case-control study of invasive cervical cancer conducted in five areas of the United States, no increased risk was noted with low intake of carotenoids, vegetables, fruits, or the vegetable subgroups rich in beta-carotene (Table 7) (Ziegler et al., 1990). The range of carotenoid intake in the study was estimated to be three- to fourfold and typical of low socioeconomic groups in the United States. Although the results of this study need to be confirmed, they suggest that carotenoids may not protect against all epithelial tumors.

To summarize, low intake of vegetables, fruits, and carotenoids is consistently associated with an increased risk of lung cancer in both prospective and retro-

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Table 7 Adjusted^a Relative Risks U.S. White Women by Carotenoid

Nutrient or food group	Highest quartile
Carotenoids	1.0
Vegetables and fruits	1.0
Fruits	1.0
Vegetables	1.0
Dark green vegetables	1.0
Dark yellow-orange vegetables	1.0

^a Adjusted for number of sexual partners, of oral contraceptive use, history of non at diagnosis, and study center.

^b Includes 271 cases and 502 controls.

Source: Adapted from Ziegler et al., 1990.

spective studies. In addition, low associated with the subsequent c planation is that beta-carotene is p manner to lung cancer risk, beta-ca that does not require its conversi other carotenoids and other cons adequately explored.

Both prospective and retrospe intake may reduce the risk of ce and less consistency among stud at present less persuasive than fo

CLINICAL TRIALS INVOLVING

A number of clinical trials of the are underway. Chemoprevention emphasize the administration of c such as beta-carotene, vitamin E, trials are currently underway wh increase carotenoid or vegetable a trials are being initiated that atte increased fiber consumption. The

Table 7 Adjusted^a Relative Risks of Invasive Squamous Cell Cervical Cancer in U.S. White Women by Carotenoid and Food Group Intake^b

Nutrient or food group	Level of consumption				<i>p</i> for trend
	Highest quartile	Quartile 3	Quartile 2	Lowest quartile	
Carotenoids	1.0	0.67	0.85	1.19	0.18
Vegetables and fruits	1.0	0.64	0.96	1.11	0.34
Fruits	1.0	1.00	0.93	1.35	0.26
Vegetables	1.0	0.92	1.16	1.16	0.43
Dark green vegetables	1.0	0.98	1.33	1.00	0.69
Dark yellow-orange vegetables	1.0	0.89	1.14	1.22	0.32

^a Adjusted for number of sexual partners, age at first intercourse, number of cigarettes/day, duration of oral contraceptive use, history of nonspecific genital infection, years since last Pap smear, age at diagnosis, and study center.

^b Includes 271 cases and 502 controls.

Source: Adapted from Ziegler et al., 1990.

spective studies. In addition, low levels of beta-carotene in blood are consistently associated with the subsequent development of lung cancer. The simplest explanation is that beta-carotene is protective. Since retinol is not related in a similar manner to lung cancer risk, beta-carotene appears to function through a mechanism that does not require its conversion to vitamin A. However, the importance of other carotenoids and other constituents of vegetables and fruits has not been adequately explored.

Both prospective and retrospective studies suggest that vegetable and fruit intake may reduce the risk of certain other cancers. Because of fewer studies and less consistency among studies, however, the epidemiological evidence is at present less persuasive than for lung cancer.

CLINICAL TRIALS INVOLVING BETA-CAROTENE

A number of clinical trials of the efficacy of beta-carotene in cancer prevention are underway. Chemoprevention clinical trials at the National Cancer Institute emphasize the administration of compounds with known antioxidant properties, such as beta-carotene, vitamin E, vitamin C, and selenium. Only a few clinical trials are currently underway which involve dietary modification designed to increase carotenoid or vegetable and fruit consumption. However, several clinical trials are being initiated that attempt to show the effects of decreased fat and increased fiber consumption. The people in these trials who adhere to a low-fat,

high-fiber diet are likely to increase their vegetable and fruit consumption as well as their intake of carotenoids, vitamin C, folacin, and other micronutrients.

SIGNIFICANCE OF FINDINGS AND IMPLICATIONS

Epidemiological studies suggest that increased vegetable and fruit consumption may reduce cancer risk. There is no evidence at present that a *moderate* increase in vegetable and fruit intake has adverse health effects. In fact, on any given day, only 83% and 59% of American adults consume at least one serving of a vegetable or fruit, respectively (Patterson and Block, 1988). While there is legitimate concern regarding the pesticide content of produce, the benefits of consuming vegetables and fruits appear to outweigh the possible risks. Furthermore, through the substitution of vegetables and fruits for other foods in an individual's diet, additional beneficial changes are likely to occur, including increased micronutrient intake; decreased calorie, fat, and sugar intake; and reduced weight. Although clinical trials to evaluate vegetable and fruit consumption directly have not been completed, it seems prudent to recommend increased intake, based on the likely benefit and unlikely risk. Thus the National Cancer Institute, the National Academy of Sciences, and the U.S. Department of Agriculture all suggest increasing vegetable and fruit consumption.

Also important is consumption of a variety of vegetables and fruits. Given the present state of knowledge, increasing only those vegetables and fruits rich in beta-carotene cannot be definitively recommended. Although beta-carotene is a plausible protective agent, other carotenoids, other micronutrients such as vitamin C or folacin, and other plant constituents such as dietary fiber or indoles may also play a role.

While beta-carotene supplements and multiple-vitamin supplements at doses comparable to the Recommended Dietary Allowances are not known to cause adverse health effects, they are not an acceptable substitute for increased vegetable and fruit intake since the protective factor(s) in vegetables and fruits has not been identified. Even if clinical trials clearly implicate beta-carotene as beneficial, vegetables and fruits could still contain additional crucial substances.

The National Cancer Institute currently suggests increasing vegetable and fruit intake to five servings a day. The recommendation is not based on epidemiological studies of cancer or overall morbidity and mortality. Instead it is derived from data describing U.S. dietary patterns and reflects what seems achievable by Americans on a Western diet and what provides adequate intake of important micronutrients. More research to clarify the health benefits of various levels of intake of vegetables and fruit would facilitate determining future guidelines. In the interim, diets oriented toward including five servings of vegetables and fruit a day are nutrient-dense and may reduce cancer risk.

Vegetables, Fruits, and Risk

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Intervention in F

INTRODUCTION

Vitamin A plays an essential role in human health (Hicks, 1983). Certain carotenoids, such as those found in red, orange, and yellow fruits and vegetables, are precursors for vitamin A (retinol) and have their own health benefits. Beta-carotene is particularly important since it can be converted in the body to vitamin A. In the natural world, carotenoids serve a similar function to vitamin A as compared with those that are not converted to vitamin A. They have a protective role in tissues with high metabolic and inorganic activity.

Epidemiological Studies

Epidemiological studies have shown that high intake of carotenoid-containing foods is associated with a lower risk of many cancers including lung, stomach, and esophagus (1975; Hirajama, 1979; Peto and Peto, 1976; Greenwald et al., 1986). I